Primary chemotherapy with gemcitabine, liposomal doxorubicin and docetaxel in patients with locally advanced breast cancer: results of a phase I trial

Peter Schmid[a], Jutta Krocker[b], Carsten-Oliver Schulz[a], Katarzyna Michniewicz[b], Annette Dieing[a], Holm Eggemann[b], Volker Heilmann[c], Jens-Uwe Blohmer[d], Orhan Sezer[a], Dirk Elling[b] and Kurt Possinger[a]

The primary objective was to determine the optimal doses for gemcitabine (prolonged infusion), liposomal doxorubicin (Myocet) and docetaxel as primary (neoadjuvant) chemotherapy for locally advanced breast cancer. Secondary objectives included evaluation of the safety and efficacy of the regimen. Patients ($n=19$) with histologically confirmed stage II or III breast cancer were treated with liposomal doxorubicin (50–60 mg/m^2) and docetaxel (60–75 mg/m^2) on day 1, and gemcitabine as 4-h infusion (350–400 mg/m^2) on day 4. Treatment was repeated every 3 weeks for a maximum of 6 cycles. The maximum tolerated doses were gemcitabine 350 mg/m^2, liposomal doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2. Dose-limiting toxicities were stomatitis, diarrhea and infection. The predominant hematologic toxicity was mild-to-moderate myelosuppression with grade 3/4 neutropenia in 20% of cycles. Non-hematologic toxicity was generally mild, with no grade 4 toxicities being observed. Predominant non-hematologic toxicity was stomatitis, which occurred in 95% of patients. Grade 3 toxicities were reported for stomatitis, nausea, diarrhea, infection and constipation. No cases of cardiac, renal, pulmonary or neurotoxicity were observed. The clinical response rate was 83% and histologically confirmed, clinically complete remissions occurred in two patients (11%). We conclude that the combination of gemcitabine (prolonged infusion), liposomal doxorubicin and docetaxel is safe and highly effective in patients with locally advanced breast cancer as defined by maximum tolerated doses. The evaluated schedule is suitable for phase II studies. *Anti-Cancer Drugs* 16:21–29 © 2005 Lippincott Williams & Wilkins.

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[a]Department of Oncology and Hematology, Charité Campus Mitte, Humboldt University Berlin, Berlin, Germany, [b]Department of Obstetrics and Gynecology, Oskar-Ziethen Hospital, Berlin, Germany, [c]Department of Obstetrics and Gynecology, University Clinical Center, Ulm, Germany and [d]Department of Obstetrics and Gynecology, Charité Campus Mitte, Humboldt University Berlin, Berlin, Germany.

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Correspondence to P. Schmid, Medizinische Klinik II, Department of Oncology and Hematology, Charité Campus Mitte, Humboldt University Berlin, Schumannstrasse 20/21, 10117 Berlin, Germany.
Tel: +49 30 450 613005; fax: +49 30 450 513952;
e-mail: peter.schmid@charite.de

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Introduction

Perioperative systemic treatment has made a major impact on relapse-free and overall survival of women with early-stage breast cancer [1,2]. Conventionally, perioperative systemic therapy is administered after local treatment. However, in recent years, the application of chemotherapy before the treatment of the primary lesions with surgery or radiation (primary systemic treatment) has become increasingly common.

Primary systemic treatment has been evaluated in a number of randomized trials comparing neoadjuvant and adjuvant anthracycline-based chemotherapy [3–7]. Although primary chemotherapy was associated with a higher rate of breast-conserving surgery, it generally failed to increase disease-free or overall survival. However, several trials showed that response to neoadjuvant chemotherapy is correlated with survival [4,6,8,9]. Patients who experience complete disappearance of their primary tumor seem to have the greatest survival advantage from neoadjuvant chemotherapy. As only a minority of patients are reported to achieve a complete response, current approaches for primary systemic therapy focus on increasing the pathologic response rate.

The role of taxanes as primary chemotherapy has been studied extensively during the last few years. Both paclitaxel and docetaxel have shown considerable activity as single-agent primary chemotherapy with response rates ranging from 3 to 25% [10–12]. In patients with stage II or IIIA breast cancer, single-agent paclitaxel has shown comparable efficacy to anthracycline-based polychemotherapy [13]. Furthermore, the sequential use of docetaxel after anthracycline-based primary chemotherapy

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has been shown to significantly increase response rates. This effect was evident both in anthracycline-sensitive and -refractory patients [14].

In combination, anthracyclines and taxanes have shown superior response rates compared to non-taxane containing anthracycline-based regimens [15–17]. The impact on disease-free and overall survival, however, has yet to be defined. Furthermore, combinations of anthracycline, taxanes and gemcitabine have shown high efficacy in metastatic breast cancer with response rates up to 92%, including 31% complete responses [18,19].

The primary objective of the current study was to determine the maximum tolerated doses (MTDs) of a regimen containing gemcitabine (as a prolonged infusion), liposomal doxorubicin and docetaxel as primary chemotherapy for locally advanced breast cancer. Secondary objectives included evaluation of safety and efficacy of the regimen.

Patients and methods

Study design

This open-label, phase I study was conducted at two centers in Germany (Berlin and Ulm) between September 2000 and November 2001. The study was conducted in accordance with the Declaration of Helsinki and ICH Harmonized Tripartite Guidelines for Good Clinical Practice, in compliance with local regulations, and with the approval of an independent ethics committee (Ethikkommission der Charite Campus Mitte).

Patient eligibility

Previously untreated patients (18–65 years) with newly diagnosed, histologically confirmed breast cancer with T2–4, N0–1, M0 disease were included in the trial. Patients were required to have adequate hematological, renal, hepatic and cardiac function [absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/l; platelets $\geq 100 \times 10^9$/l; hemoglobin ≥ 8 g/dl; serum bilirubin $< 1.5 \times$ upper limit of normal (ULN); serum transaminase $< 2.5 \times$ ULN; serum creatinine within normal range; left ventricular ejection fraction (LVEF) $\geq 50\%$], a Karnofsky performance status ≥ 70, a negative pregnancy test, absence of lactation, and appropriate contraception throughout the study (in premenopausal women only).

Exclusion criteria included previous systemic or local treatment for breast cancer (including surgery, radiotherapy, cytotoxic and endocrine treatments), evidence of distant metastases, a history of other malignancies (except for curatively treated non-melanoma skin cancer or *in situ* carcinoma of the cervix), pre-existing peripheral neuropathy greater than grade 1 [NCI Common Toxicity Criteria (CTC)], cardiac arrhythmias greater than Lown II, congestive heart failure, active infection, or any other serious underlying medical or psychiatric condition which would impair the ability of the patient to receive protocol treatment.

Written, informed consent was obtained from all patients.

Treatment plan

Eligible patients received up to six 3-week cycles of primary chemotherapy with liposomal doxorubicin, docetaxel and gemcitabine. In each cycle, liposomal doxorubicin (50–60 mg/m^2, i.v. infusion over 60 min) was administered first on day 1, followed by docetaxel (60–75 mg/m^2, i.v. infusion over 60 min) on the same day. Gemcitabine (350–400 mg/m^2, i.v. infusion over 4 h) was administered on day 4.

All patients were given prophylactic corticosteroid premedication (oral dexamethasone 8 mg), before (12 and 1 h) and after (12, 24 and 36 h) docetaxel infusion. Standard antiemetic therapy was administered as needed on a prophylactic or treatment basis in compliance with the standards of the center. Recombinant granulocyte colony-stimulating factor (G-CSF; 5 μg/kg, s.c.) was given on days 5–12 or until ANC $> 1.5 \times 10^9$/l.

Doses were assigned at registration according to a dose escalation scheme (Table 1) and were not escalated intraindividually. Dose escalation and determination of the MTD were based on the occurrence of dose-limiting toxicities (DLT) in cycle 1 only. DLTs were defined as (i) any grade 3 or 4 non-hematologic toxicity (other than nausea, vomiting or alopecia); (ii) febrile neutropenia, i.e. ANC < 1000/μl concurrent with fever (defined as one oral temperature measurement of ≥ 38.5°C or three oral temperature measurements of ≥ 38.0°C in a 24-h period); (iii) grade 4 thrombocytopenia or thrombocytopenia of any grade associated with bleeding; and (iv) any > 2 grade toxicity (other than nausea, vomiting, alopecia or anemia) that persisted beyond day 35 after the start of cycle 1. At least three patients assessable for toxicity were treated at each dose level. If none of the first three patients experienced DLT, the next dose level was initiated. If DLT occurred in any of the first three patients, two additional patients were treated at the same level. Dose escalation was continued if DLT was observed in a maximum of two patients of the expanded cohort. When DLT occurred in three or more of five patients, dose escalation was stopped and the next set of patients was treated at the previous level. The MTD was confirmed if DLT occurred in less than three of eight or less than four of 10 patients treated at this dose level. Patients experiencing DLT were continued on treatment at the next lower dose level or withdrawn from the trial.

Dose reductions were not allowed during the first cycle. For subsequent cycles, dose modifications were planned

Table 1 Dose escalation and dose-limiting toxicities

Dose level	Gemcitabine (mg/m^2)	Liposomal doxorubicin (mg/m^2)	Docetaxel (mg/m^2)	*n*	Cycles (range per patient)	DLT	Type of DLT
I	350	50	60	3	18 (6)	0	–
II	350	50	75	5	28 (4–6)	0	–
III	350	60	75	8	40 (2–6)	0	–
IV	400	60	75	3	15 (3–6)	3	grade 3 diarrhea and grade 3 infection ($n=2$); grade 3 stomatitis ($n=1$)
Total				19	101 (2–6)	3	–

for severe toxicity. Doses of gemcitabine, liposomal doxorubicin and docetaxel were to be reduced by one dose level if ANC $< 500/\mu l$ for more than 7 days, ANC $< 100/\mu l$ for more than 3 days, there was an episode of febrile neutropenia or grade 3 gastrointestinal side-effects. If grade 2 elevations of serum transaminase levels occurred, gemcitabine was reduced by one dose level while liposomal doxorubicin and docetaxel remained unchanged. In case of grade 3 elevations of serum transaminase levels, treatment with gemcitabine was discontinued. If grade 2 neurotoxicity was experienced, docetaxel was reduced by one dose level. For grade 3 neurotoxicity, docetaxel was discontinued.

Treatment was planned for 6 cycles unless there was evidence of unacceptable toxicity, disease progression or inadequate efficacy (defined as a decrease in tumor size $< 25\%$ after 2 courses or $< 50\%$ after 4 courses) or if the patient requested to be released. A new cycle was only started if ANC was $\geq 1.5 \times 10^9/l$, platelet count was $\geq 100 \times 10^9/l$ and the non-hematologic toxicity grade was ≤ 1 (with the exception of alopecia, nausea and vomiting). If a delay of treatment became necessary, blood counts were repeated every 2 days, so that treatment could be continued as soon as possible. If treatment had to be delayed for more than 2 weeks, the patient was withdrawn from the study. Treatment was also discontinued if resting LVEF decreased by ≥ 20 points from baseline to final values of LVEF $\geq 50\%$ of starting values or ≥ 10 points from baseline to a final LVEF value $< 50\%$ of starting value, there was evidence of symptomatic arrhythmia or second degree atrioventricular block, there was any grade 3 non-hematologic toxicity except for nausea, vomiting, alopecia, infection and gastrointestinal side-effects or any grade 4 non-hematologic toxicity.

Patients were scheduled to undergo surgery within 28 days after the start of the last chemotherapy cycle. Surgery was performed earlier in case of disease progression, inadequate efficacy ($< 25\%$ after 2 courses or $< 50\%$ after 4 courses), complete clinical response or prolonged toxicity. Surgery consisted of either a modified radical mastectomy or breast-conserving surgery in order to provide tumor-free margins of at least 1 cm. Standard axillary lymph node surgery with excision of ≥ 10 lymph nodes was to be performed in all cases. Patients who underwent breast-conserving surgery received adjuvant irradiation of the breast.

Patient evaluation and follow-up

Before entry into the study, all patients underwent staging work-up including a complete history and physical examination, complete blood count, chemistry profile, chest X-ray, abdominal ultrasound and/or a computed tomography, bone scan, and mammograms of both breasts. Cardiac function was assessed by electrocardiogram (ECG) and measurement of LVEF by bidimensional echocardiography (ECHO). Primary tumor size and axillary lymph node involvement were assessed by clinical, X-ray and sonographic evaluation. In patients with unclear findings primary tumor size was also assessed by magnetic resonance (MR) mammography. Histologic confirmation of the invasive tumor was performed by core needle biopsy. Clinical tumor size and nodal status were assessed by palpation before each cycle of chemotherapy, and by ultrasound (and MR mammography in case of unclear findings) after 2, 4 and 6 courses, respectively. The product of the two greatest perpendicular diameters was used to compare tumor size before and after chemotherapy.

A clinical complete response (cCR) was considered a complete disappearance of all clinically detectable malignant disease by palpation as well as imaging techniques. Clinical partial response was defined as a $\geq 50\%$ decrease in total tumor size (PR). A decrease in tumor size of $< 50\%$ or an increase of $< 25\%$ was classified stable disease (SD). An increase of $\geq 25\%$ in tumor size at any time was considered to be progressive disease (PD). In patients with clinically negative nodes at study entry, the development of palpable nodes during preoperative chemotherapy was considered evidence of PD.

Surgical specimens were evaluated for pathologic tumor status. Samples with no histologic evidence of invasive tumor at the primary site and in the axillary lymph nodes

were classified as pathologic complete responses (pCR). Core biopsies and surgical specimens were reviewed centrally by a pathologist at the Humboldt University of Berlin.

Adverse events and toxicities were recorded for every cycle. They were graded using the NCI CTC (version 2.0). Cardiac function was monitored after every second cycle by ECG and assessment of the LVEF by ECHO.

During follow-up, a clinical assessment for each patient was performed at 3-month intervals for the first 3 years and every 6 months thereafter. In addition to the routine physical examination (paying particular attention to local recurrence and lymph nodes) performed at each visit, mammography was performed every 6 months during the first 2 years and once a year thereafter to the involved breast and once a year to the contralateral breast. Cardiac function was monitored every 6 months by LVEF assessment and ECG.

Statistical analysis

The number of patients included in the study was based on the need to establish the MTD of the gemcitabine/liposomal doxorubicin/docetaxel regimen as primary chemotherapy for locally advanced breast cancer. Standard descriptive methods were applied for analyses.

Results

Patient characteristics

Between September 2000 and July 2001, 19 patients with newly diagnosed, locally advanced breast cancer were enrolled. All patients were assessable for safety analysis and 18 patients were assessable for efficacy. Patient baseline characteristics are listed in Table 2. All patients were Caucasian and more than half had clinical stage IIIB disease, including five patients with inflammatory breast cancer. Axillary lymph nodes were palpable in 84% of patients.

Chemotherapy administration

A total of 101 evaluable cycles were administered during the study. All patients received ≥ 2 cycles and the mean number of cycles per patient was 5.3. One patient was withdrawn after 2 cycles because of progressive disease. In another two patients, primary chemotherapy was stopped after 4 courses due to inadequate response (achieved $< 50\%$ reduction in tumor size). One patient at dose level IV was withdrawn after 3 courses for toxicity. The remaining patients received the intended maximum number of 6 courses. The median cumulative doxorubicin dose was 300 mg/m^2 (120–360 mg/m^2). Dose reductions were required in six patients (dose levels II, III and IV). Reasons for dose reduction were grade 3 stomatitis ($n = 3$), grade 3 diarrhea in association with infection ($n = 2$) and grade 3 elevation of serum γ-glutamyl

Table 2 Patient baseline characteristics

Dose level	I	II	III	IV	Total
n	3	5	8	3	19
Age (years) [mean (range)]	63.0 (56–64)	45.0 (35–62)	55.5 (31–61)	54.0 (38–63)	50.6 (31–64)
Initial clinical tumor size [*n* (%)]					
T2	1	3	2	0	6 (32)
T3	1	0	1	2	4 (21)
T4a–c (non-inflammatory)	1	1	2	0	4 (21)
T4d (inflammatory breast cancer)	0	1	3	1	5 (26)
Initial clinical nodal status [*n* (%)]					
N0	1	1	1	0	3 (16)
N1/2	2	4	7	3	16 (84)
Initial tumor stage [*n* (%)]					
IIA	1	0	1	0	2 (11)
IIB/IIIA	1	3	2	1	7 (37)
IIIB	1	2	5	2	10 (53)
Menopausal state [*n* (%)]					
pre- and perimenopausal	0	1	3	1	5 (26)
postmenopausal	3	4	5	2	14 (74)
Hormone receptor status [*n* (%)]					
ER and/or PgR positive	2	3	6	1	12 (63)
ER and PgR negative	0	2	0	1	3 (16)
not available	1	0	2	1	4 (21)
HER2/*neu* [*n* (%)]					
score 0–2	0	4	3	1	8 (42)
score 3	1	1	3	1	6 (32)
not available	2	0	2	1	5 (26)
Histology [*n* (%)]					
ductal carcinoma	3	4	6	2	15 (79)
lobulary carcinoma	0	1	1	1	3 (16)
not further specified	0	0	1	0	1 (5)
Histological grade [*n* (%)]					
1	0	0	1	0	1 (5)
2	1	2	5	2	10 (53)
3	2	3	2	1	8 (42)

ER = estrogen receptor; PgR = progesterone receptor.

transpeptidase ($n = 1$). Most treatment cycles (97%) were administered every 3 weeks. Treatment delays were a result of persistent mucositis on day 22 ($n = 2$) and patient's request ($n = 1$). The median relative dose intensities for the combination was 0.96 (Table 3). At the MTD, the median relative dose intensities for gemcitabine, doxorubicin and docetaxel were 0.98, 0.97 and 0.97, respectively.

Dose escalation and DLTs

Sixteen patients received a total of 86 courses at dose levels I to III with no dose-limiting events (Table 1). At dose level IV, however, three of three patients treated developed DLT during the first course. DLTs consisted of grade 3 diarrhea in two patients in association with febrile neutropenia, respectively. A third patient experienced grade 3 stomatitis. In all patients, DLT toxicities resolved completely by day 28 after the start of the first cycle. Treatment was continued in all patients experiencing DLT after dose reduction by one dose level. The MTD was 350 mg/m^2 gemcitabine, 60 mg/m^2 liposomal doxorubicin and 75 mg/m^2 docetaxel.

Toxicity

The tolerability and toxicity of the regimen was evaluated in 101 documented cycles. Toxicities are listed in Tables 4 and 5 using the worst toxicity on study for individual patients. The combination was generally well tolerated. Fourteen patients (74%) completed the planned 6 cycles of chemotherapy. One patient withdrew from the study earlier than expected because of toxicity (grade 3 diarrhea in association with severe colitis). The remaining discontinuations were as a result of disease progression or inadequate tumor regression.

Despite prophylactic application of G-CSF, the predominant hematological toxicity was mild-to-moderate neutropenia, which was generally uncomplicated and rapidly reversible (Table 4). Anemia was frequent but mild, with no grade 3 or 4 toxicity. Thrombocytopenia was noted in 47% of patients, but was grade 3/4 in one patient only.

Non-hematologic toxicities were usually mild (Table 5) with no grade 4 episodes reported. Grade 3 toxicities were reported for stomatitis (three patients, 6% of

Table 3 **Mean actual doses, cumulative doses and relative dose intensity**

Dose level	Gemcitabine (mg/m^2)			Doxorubicin (mg/m^2)			Docetaxel (mg/m^2)			Combination
	Actual dose	Cumulative dose	Relative dose intensity	Actual dose	Cumulative dose	Relative dose intensity	Actual dose	Cumulative dose	Relative dose intensity	Relative dose intensity
I	350	2100	1.0	50	300	1.0	60	360	1.0	1.0
II	345	1930	0.98	49	274	0.98	73	411	0.98	0.98
III	341	1706	0.98	58	291	0.97	72	362	0.97	0.97
IV	360	1683	0.90	52	243	0.87	63	295	0.84	0.87

Table 4 **Maximum individual and cumulative hematologic toxicity**

Dose level	CTC grade	Leukocyte		Neutrophil		Platelet		Hemoglobin	
		Individual	Cumulative (%)	Individual	Cumulative (%)	Individual	Cumulative (%)	Individual	Cumulative (%)
I	0	0	6 (33.3)	0	7 (38.9)	1	11 (61.1)	1	9 (50.0)
	I	0	2 (11.1)	1	2 (11.1)	0	5 (27.8)	0	7 (38.9)
	II	1	3 (16.7)	0	2 (11.1)	1	1 (5.6)	2	2 (11.1)
	III	1	4 (22.2)	1	3 (16.7)	1	1 (5.6)	0	0 (0.0)
	IV	1	3 (16.7)	1	4 (22.2)	0	0 (0.0)	0	0 (0.0)
II	0	0	12 (42.9)	1	15 (50.0)	1	22 (78.6)	0	18 (64.3)
	I	1	3 (10.7)	0	2 (7.1)	2	4 (14.3)	5	10 (35.7)
	II	1	7 (25.0)	1	6 (21.4)	2	2 (7.1)	0	0 (0.0)
	III	3	6 (21.4)	3	6 (21.4)	0	0 (0.0)	0	0 (0.0)
	IV	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
III	0	1	26 (65.0)	3	29 (72.5)	6	36 (90.0)	4	33 (82.5)
	I	3	7 (17.5)	0	3 (7.5)	1	2 (5.0)	4	7 (17.5)
	II	0	2 (5.0)	1	3 (7.5)	1	2 (5.0)	0	0 (0.0)
	III	1	2 (5.0)	0	1 (2.5)	0	0 (0.0)	0	0 (0.0)
	IV	3	3 (7.5)	4	4 (10.0)	0	0 (0.0)	0	0 (0.0)
IV	0	0	10 (71.4)	0	10 (71.4)	2	13 (92.9)	1	10 (71.4)
	I	1	2 (14.3)	1	2 (14.3)	1	1 (7.1)	2	4 (28.6)
	II	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
	III	1	1 (7.1)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
	IV	1	1 (7.1)	2	2 (14.3)	0	0 (0.0)	0	0 (0.0)
Total [*n* (%)]	0	1 (5.3)	54 (54.0)	4 (21.1)	60 (60.0)	10 (52.6)	82 (82.0)	6 (31.6)	70 (70.0)
	I	5 (26.3)	14 (14.0)	2 (10.5)	9 (9.0)	4 (21.1)	12 (12.0)	11 (57.9)	28 (28.0)
	II	2 (10.5)	12 (12.0)	2 (10.5)	11 (11.0)	4 (21.1)	5 (5.0)	2 (10.5)	2 (2.0)
	III	6 (31.6)	13 (13.0)	4 (21.1)	10 (10.0)	1 (5.3)	1 (1.0)	0 (0.0)	0 (0.0)
	IV	5 (26.3)	7 (7.0)	7 (36.8)	10 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 5 **Maximum individual non-hematologic toxicity**

Dose level	CTC grade	Nausea and vomiting	Stomatitis	Diarrhea	Constipation	Fever	Infection	Dyspnea	Asthenia	Alopecia	Skin
I	0	1	0	1	1	2	1	2	0	0	2
	I	1	1	1	0	1	1	1	2	0	1
	II	1	2	1	2	0	1	0	1	3	0
	III	0	0	0	0	0	0	0	0	–	0
	IV	0	0	0	0	0	0	0	0	–	0
II	0	0	0	4	3	1	2	3	0	0	2
	I	2	0	1	1	4	2	1	4	0	2
	II	3	4	0	1	0	1	1	1	5	1
	III	0	1	0	0	0	0	0	0	–	0
	IV	0	0	0	0	0	0	0	0	–	0
III	0	0	1	5	3	6	7	5	1	0	8
	I	5	3	1	3	1	1	2	6	0	0
	II	3	3	2	2	1	0	1	1	8	0
	III	0	1	0	0	0	0	0	0	–	0
	IV	0	0	0	0	0	0	0	0	–	0
IV	0	0	0	0	2	1	1	3	1	0	3
	I	0	0	0	0	0	0	0	1	0	0
	II	1	2	1	0	2	0	0	1	3	0
	III	2	1	2	1	0	2	0	0	–	0
	IV	0	0	0	0	0	0	0	0	–	0
Total [*n* (%)]	0	1 (5)	1 (5)	10 (52)	9 (47)	10 (53)	11 (58)	13 (68)	2 (11)	0 (0)	13 (68)
	I	8 (42)	4 (21)	3 (16)	4 (21)	6 (32)	4 (21)	4 (21)	13 (68)	0 (0)	5 (26)
	II	8 (42)	11 (58)	4 (21)	5 (26)	3 (16)	2 (11)	2 (11)	4 (21)	19 (100)	1 (5)
	III	2 (11)	3 (16)	2 (11)	1 (5)	0 (0)	2 (11)	0 (0)	0 (0)	–	0 (0)
	IV	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–	0 (0)

cycles), nausea (two patients, 5% of cycles), diarrhea (two patients, 3% of cycles, dose level IV), infection (two patients, 3% of cycles, dose level IV) and constipation (one patient, 1% of cycles, dose level IV). Pronounced alopecia was noted in all patients. No relevant infections were observed at dose levels I, II or III. Two patients at dose level IV experienced grade 3 infection (febrile neutropenia, colitis) requiring i.v. antibiotics and/or hospitalization. Predominant and dose-limiting non-hematologic toxicity was mucositis with 95% of patients experiencing stomatitis and 48% reporting diarrhea. Although generally mild-to-moderate, stomatitis affected 77% of courses and had a considerable effect on quality of life. Mucositis was the most frequent cause for delay of treatment or dose reduction.

Elevations of serum transaminase or alkaline phosphatase levels were observed in five patients (31% of courses) and were graded as severe in one patient. Hepatotoxicity resolved in all patients after discontinuation of the treatment with gemcitabine and was clinically not significant. Mild-to-moderate nausea and vomiting (grade 1 and 2) were reported in most patients (16 patients; 58% of courses). Two patients at dose level IV developed grade 3 nausea and vomiting despite prophylactic administration of parenteral antiemetics. However, nausea and vomiting remained under control with a combination of 5-HT_3 antiemetics and corticosteroids. Six patients at dose levels I–III developed a transient, mild erythematous, maculopapular rash. This rash usually developed 2–3 days after administration of gemcitabine. Specific treatment or dose reductions were not required. Despite continuation of therapy the rash resolved and did not reappear in most cases. No cases of palmar–plantar erythrodysesthesia were observed throughout the study. Transient febrile episodes occurring 12–24 h after the administration of gemcitabine were encountered in six patients (9% of courses) and were common at all dose levels. These episodes were easily managed with non-steroidal anti-inflammatory drugs, occurred most frequently following the first dose of gemcitabine, and did not require dose modifications.

No cases of cardiac, renal, pulmonary or neurotoxicity were reported throughout the study.

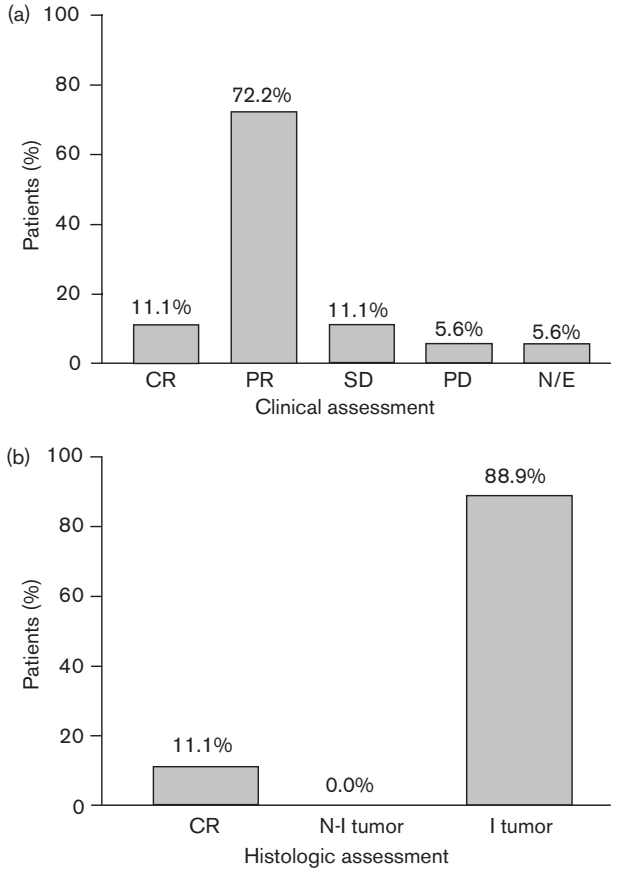
Efficacy

Eighteen patients were evaluable for efficacy. The overall clinical response rate assessed by sonographic evaluation was 83% (Fig. 1). Postoperative histologic evaluation confirmed complete disappearance of the tumor in the two patients with clinical complete responses.

Locoregional and adjuvant treatment

Breast-conserving surgery was performed in five patients. One patient with stage IIIB breast cancer did not receive surgical treatment. The remaining 13 patients received modified mastectomies. Of 16 patients with clinical lymph node involvement at the beginning of the study, 13 patients had clinically uninvolved lymph nodes after primary chemotherapy. On histologic evaluation, five patients were node-negative.

Fig. 1



(a) Clinical and (b) histological responses to chemotherapy. CR = Complete response; PR = partial response; SD = stable disease; PD = progression; N/E = not evaluable; N-I Tumor = non-invasive tumor; I Tumor = invasive tumor.

Seventeen of 19 patients received adjuvant treatment according to the protocol. Chemotherapy was administered in 14 patients. All patients with hormone receptor-positive tumors received endocrine treatment. Radiotherapy was administered in accordance with the protocol.

Discussion

The current study has provided evidence that the combination of gemcitabine (as a prolonged infusion), liposomal doxorubicin (Myocet) and docetaxel is a safe and effective regimen for primary chemotherapy in patients with locally advanced breast cancer.

Combinations of anthracyclines, taxanes and gemcitabine have shown high efficacy in metastatic breast cancer with mild non-hematologic and moderate hematologic toxicity [18,19]. In the first reported trial on a triplet combination, 36 patients with metastatic breast cancer were treated with paclitaxel (175 mg/m^2, day 1), epirubicin (90 mg/m^2, day 1) and gemcitabine (1000 mg/m^2, day 1 and 4) in 3-week intervals. The overall response rate was 92%, including 31% complete responses. Based on these results, the current trial was initiated to evaluate a modified regimen with docetaxel, liposomal doxorubicin and gemcitabine (as infusion over 4 h), for primary systemic treatment in patients with locally advanced breast cancer.

The current regimen differed in several aspects from previous combination regimens using anthracyclines, taxanes and gemcitabine. First, a liposomal formulation of doxorubicin was selected to replace conventional anthracyclines. The liposomal formulation has been shown to be effective at equivalent doses to doxorubicin but to have a significantly lower risk of cardiotoxicity [20,21]. Second, gemcitabine was administered over 4 h instead of the standard 30 min. The rationale for this approach was a potential higher efficacy compared with the shorter-term application. Gemcitabine is a pro-drug that has to be phosphorylated intracellularly to gemcitabine triphosphate (dFdCTP) in order to exhibit its antineoplastic activity [22–24]. The rate-limiting enzyme in the formation of dFdCTP is deoxycytidine kinase, which is saturated at concentrations following a 30-min infusion of gemcitabine [25,26]. Hence, optimal accumulation of dFdCTP is dependent upon achieving plasma concentrations of gemcitabine that use the full capacity of the deoxycytidine kinase without exceeding the saturation concentration. Pharmacological studies indicate that higher intracellular concentrations of active metabolites can be achieved by prolongation of the administration of gemcitabine [25,27]. Furthermore, phase I and II trials using gemcitabine as a 3-, 4- or 6-h infusion showed that the increased infusion duration is associated with a considerably lower MTD ranging from 250 to 450 mg/m^2 [28–31]. Toxicity profiles were comparable to the side-effects seen with standard application of gemcitabine. Other trials, using gemcitabine at a fixed dose rate of 10 mg/m^2/min, reveal higher MTDs. However, these MTDs are associated with considerably more side-effects, especially pronounced hematologic toxicities [32–34] and did not suggest a clear advantage in efficacy.

The current treatment plan consisted of docetaxel and liposomal doxorubicin administered on day 1 and gemcitabine administered on day 4. This strategy was developed considering potential time- and schedule-dependent interactions between the three agents. *In vitro* studies indicate an antagonistic cytotoxic effect of gemcitabine if it is given less than 24 h after paclitaxel, an effect attributable to a temporary cell cycle arrest in the G_2/M phase. In contrast, if gemcitabine is administered 48–72 h after taxane treatment, a synergistic effect can be observed between gemcitabine and paclitaxel. At

this time tumor cells have been shown to progress to G_1/S phase again [35].

The incidence of grade 3/4 hematologic side-effects (neutropenia 20%, leukopenia 20%, platelets 1%, anemia 0%) was markedly lower than previously reported for combinations of anthracyclines, taxanes and gemcitabine. In a recent phase III trial in metastatic breast cancer, for example, 92.7, 74.8 and 28.5% of patients receiving gemcitabine, epirubicin and paclitaxel experienced grade 3 or 4 neutropenia, leukopenia and thrombocytopenia, respectively [36]. Similar results have been reported from other trials, with up to 19% of patients experiencing febrile neutropenia [37–40]. The low degree of leukopenia and neutropenia in the presented trial can probably be mainly attributed to the prophylactic use of G-CSF. However, other factors might have contributed to the low rate of hematologic toxicity. Liposomal doxorubicin has been reported to cause significantly less neutropenia than conventional doxorubicin in combination therapy [21]. Furthermore, prolonged infusion of gemcitabine has been proposed to cause less platelet toxicity than standard infusional therapy [31]. The relatively mild hematologic toxicity of the current regimen is also reflected in a high mean relative dose intensity of 97% at the MTD. At this dose level, dose reductions were required in two patients and 7 cycles only. In contrast, in the above-mentioned randomized trial, 22 and 8% of courses had to be delayed or reduced, respectively [36].

The majority of reported non-hematologic adverse events were mild-to-moderate in intensity. The predominant and dose-limiting non-hematologic toxicity was mucositis which was grade 3 in 6% of courses. Similar incidences and acute toxicity profiles have been reported in other trials using combinations of anthracyclines, taxanes and gemcitabine [18,19,37–40]. Stomatitis was generally manageable without specific treatment or temporary treatment interruption.

In addition to a favorable toxicity profile, the presented regimen demonstrated high efficacy. The clinical response rate as assessed by sonographic measurement was 83%. Histologic evaluation confirmed a complete response in two patients including one patient with inflammatory breast cancer. These results are confirmed by preliminary data from four phase II trials evaluating combinations of epirubicin, gemcitabine and paclitaxel or docetaxel, respectively [37–40]. In these trials, clinical response rates were 83–96% and pCR rates were 22–25%. However, the final results of these studies need to be assessed before definite conclusions can be drawn.

In summary, the results of the presented study show that the combination of gemcitabine as prolonged infusion, liposomal doxorubicin and docetaxel is a safe and highly active regimen for primary chemotherapy in patients with locally advanced breast cancer. The maximum tolerated doses were gemcitabine 350 mg/m^2, liposomal doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2, with stomatitis, diarrhea and infection being dose limiting. The regimen is suitable for further evaluation. A phase II study has been initiated.

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